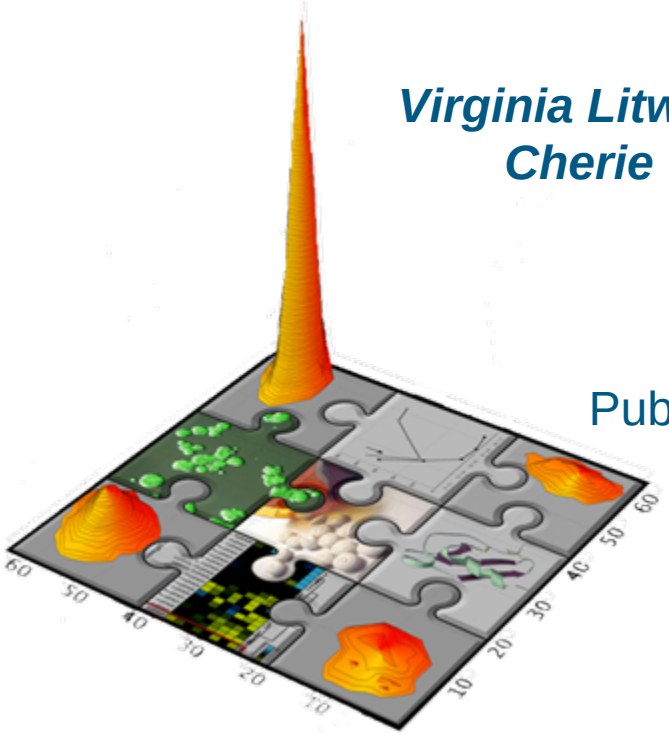
The Role of Biomarkers in Clinical Trials and The Fit-for-Purpose Method Validation Approach

Virginia Litwin, Ph.D., *Principal Scientist, Covance*
Cherie Green, *Principal Scientist, Amgen*



Public Workshop - Clinical Flow Cytometry in Hematologic Malignancies, Silver Springs, MD

February 25-26, 2013

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Presentation Overview

1. The Role of Biomarkers in Clinical Trials
2. The Fit-for-Purpose Method Validation Approach
3. Applying Fit-for-Purpose Method Validation Approach to Flow Cytometric Methods
4. System Validation

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Biomarker

A characteristic that is measured and evaluated as an indicator of:

- normal biologic processes
- pathogenic processes
- pharmacologic responses to a therapeutic intervention

Clin Pharmcol Ther 69:89, 2001

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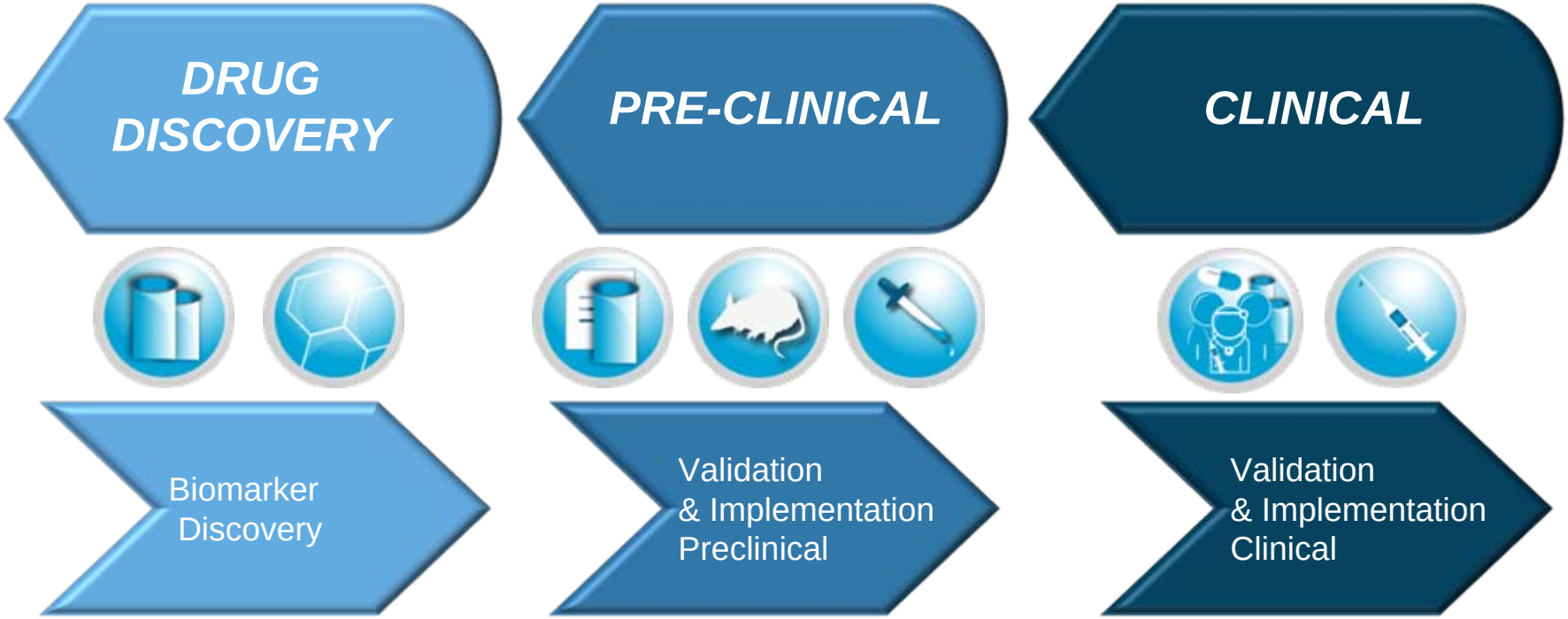
Use of Biomarkers in Drug Development



- Target Identification
- Drug Screening
- Candidate Selection
- Lead Characterization

- MOA
- *In vivo* POC
- Toxicity
- Potency
- Certificate of Analysis

- Exploratory Biomarker
- PD Biomarker
- Efficacy
- Safety/Toxicity
- Enrollment
- Companion Diagnostic

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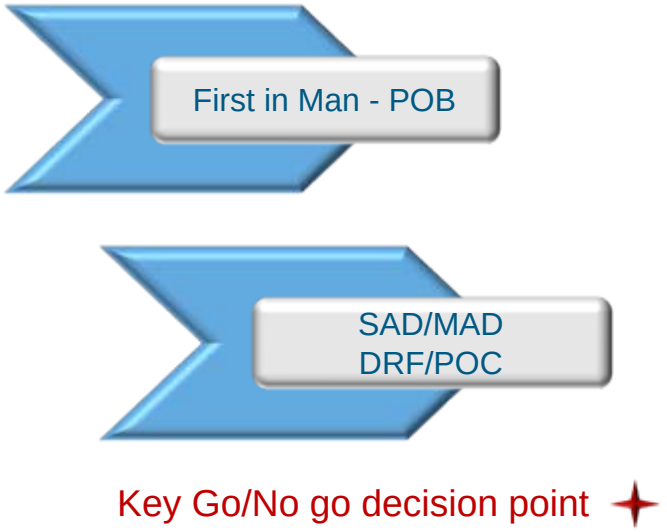
Intended Use of the Data

Early Phase Clinical Trials

- Exploratory/Translation Medicine Biomarker
- PD Biomarker
- Functional Response Biomarker
- Toxicity/Safety Biomarker
- Purpose of Biomarker Data
  - **Go/No-Go Decision-Making Tool**
  - Support Proof-of-Concept
  - **Dose Selection**



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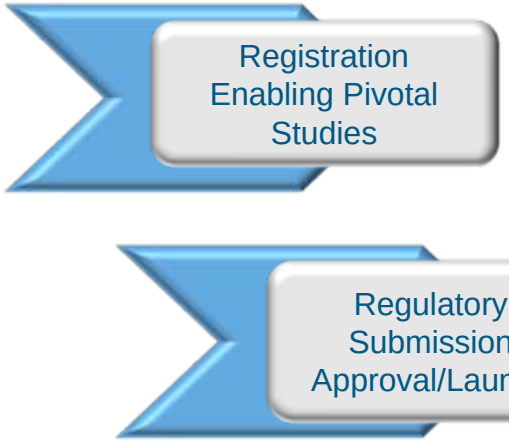
Intended Use of the Data

Late Phase Clinical Trials

- Disease Biomarkers
- Pharmacogenomic Biomarkers (PGx)
- Purpose of Biomarker Data
  - Provide a better understanding of inter-patient variability in response
  - **Enhance overall quality and scientific strength of regulatory dossiers (label claims)**
  - Companion diagnostic



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Fit-For-Purpose Method Validation

Pharmaceutical Research, Vol. 22, No. 4, April 2005 (© 2005)
10.1007/s11095-005-2495-9

Conference Report

Method Validation and Measurement of Biomarkers in Nonclinical and Clinical Samples in Drug Development: A Conference Report

Jean W. Lee,[1,17] Russ S. Weiner,[2] Jeff M. Sailstad,[3] Ronald R. Bowsher,[4] Dean W. Knuth,[5] Peter J. O'Brien,[6] Jean L. Fourcroy,[7] Rakesh Dixit,[8] Lini Pandite,[9] Robert G. Pietrusko,[10] Holly D. Soares,[11] Valerie Quarmby,[12] Ole L. Vesterqvist,[2] David M. Potter,[11] James L. Witliff,[13] Herbert A. Fritche,[14] Timothy O'Leary,[15]

Pharmaceutical Research, Volume 23, No. 2, February 2006 (© 2006)
DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

Jean W. Lee,[1,16,17] Viswanath Devanarayan,[2] Yu Chen Barrett,[3] Russell Weiner,[3] John Allinson,[4] Scott Fountain,[5] Stephen Keller,[6] Ira Weinryb,[7] Marie Green,[8] Larry Duan,[9] James A. Rogers,[10] Robert Millham,[10] Peter J. O'Brien,[11] Jeff Sailstad,[12] Masood Khan,[13] Chad Ray,[14] and John A. Wagner[15]

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Driving Force for the Papers

Who

- Generated by the AAPS, Bio-Tec Section, Ligand Binding Assay Bioanalytical Focus

Why

- Usage of biomarker data was impeded by a lack of understanding on how to interpret the data
- Application of existing validation paradigms to were not appropriate to biomarker research
  - Can’t apply one set of rules to all technologies

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Fit-For-Purpose

Fit

- Biomarker data must be reliable and accurate data

Purpose

- Decision making during drug development

Fit-for-Purpose

- Analytical validation requirements are specific to the stage of drug development
- Consideration to the **intended use** of the biomarker data
- Consideration to the regulatory requirements associated with that use
- Practical, iterative approach

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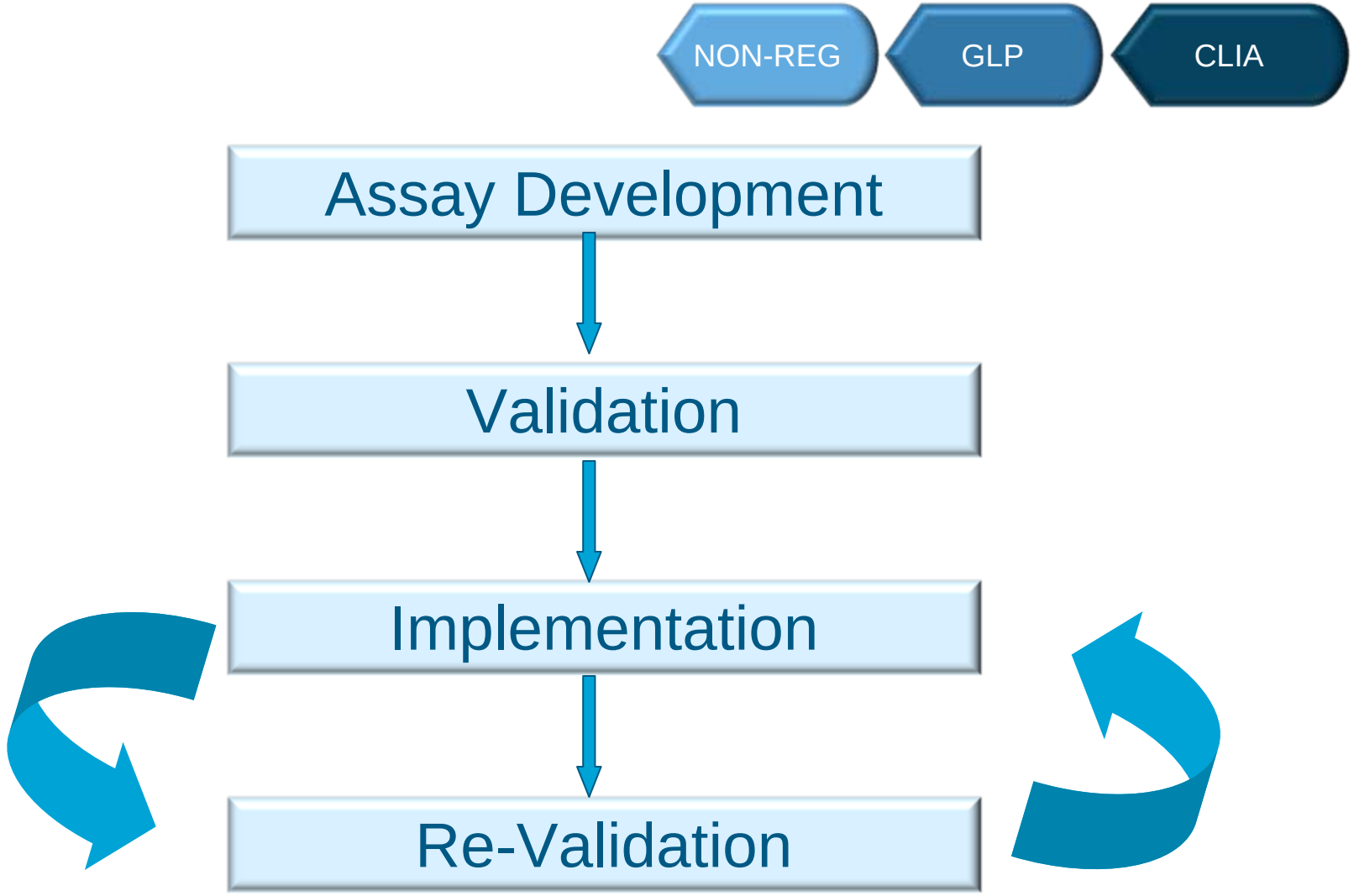
Iterative Approach



Fit-for-Purpose Approach in Flow Cytometry

Research paper

Recommendations for the validation of flow cytometric testing during drug development: I instrumentation

Cherie L. Green[a]*, Lynette Brown[b], Jennifer J. Stewart[b], Yuanxin Xu[c], Virginia Litwin[d], Thomas W. McCloskey[e]

Recommendations for the validation of flow cytometric testing during drug development: II assays

Denise M. O'Hara[a], Yuanxin Xu[b], Zhiyan Liang[c], Manjula P. Reddy[d], Dianna Y. Wu[e], Virginia Litwin[f],*

JIM, 363:104-119, 2011
JIM, 363:120-134, 2011

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Driving Force Behind the Papers

Who

- Generated by the AAPS, Bio-Tec Section, Ligand Binding Assay Bioanalytical Focus, **Flow Cytometry Action Programming Committee**

Why

- Flow cytometric methods can be more challenging to validate that other technologies

Analytical issues

- Cellular measurands
- Lack of cellular reference material
- Highly complex reagents
  - mAb, fluorescent tags, tandem dyes
- Highly complex instrumentation

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Considerations for Fit-for-Purpose Validation

- Methodology
- Sample Type
- Data Type

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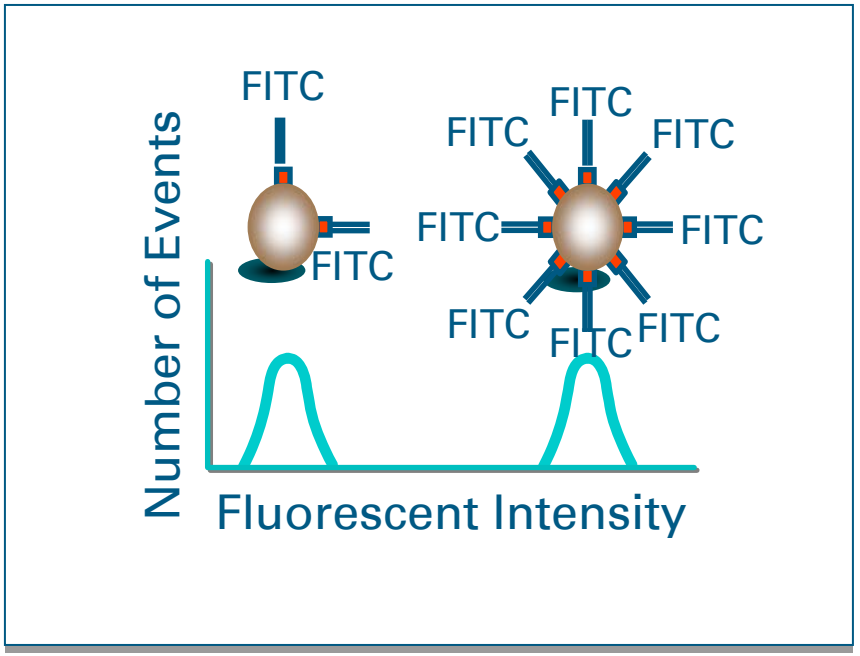
Validation Considerations Flow Cytometry

Data Type/Bioanalytical Category

- Quasi-quantitative
  - Possess certain attributes
- Results are numeric and expressed in terms of a characteristic of the test sample
  - anti-drug antibody assays (where the readout is a titer or % bound)
  - enzymatic assays (where activity might be expressed per unit volume)
  - flow cytometric assays
- No reference standard



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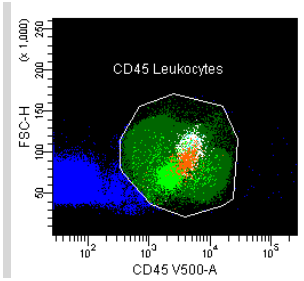
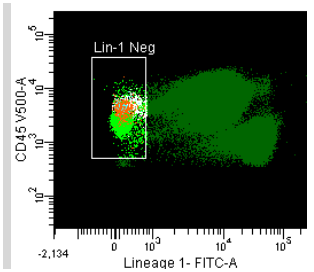
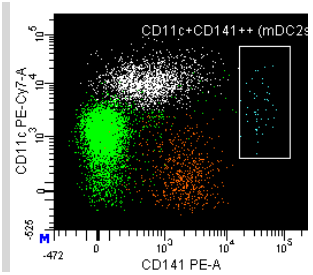
Validation Considerations Flow Cytometry

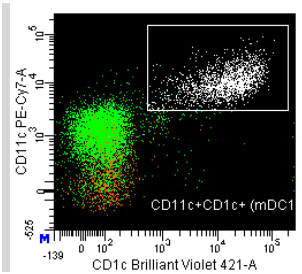
Data Type/Assay Complexity

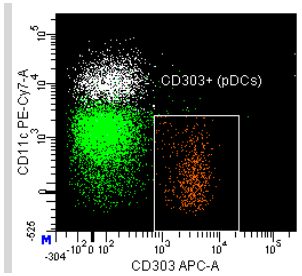
- Surface phenotyping
  - Simple/complex (IVD/RUO)
  - Quantitative antigen expression (MESF/ABC)
- Intracellular
  - Cytokines
  - Nuclear proteins
  - Phosphorylated antigen detection (phosphoflow)
- Neutralizing antibody (NAb), Anti-drug Antibody (ADA)
- Receptor Occupancy
- Nucleic acid detection
  - Cell cycle
  - Apoptosis











Validation Considerations Flow Cytometry

Validation Stringency & Regulatory Requirements

- GLP, CLIA, GMP
- Establish the intended use of the data
  - Exploratory biomarker
  - PD biomarker
  - Safety
  - Enrollment biomarker
  - Companion Diagnostic

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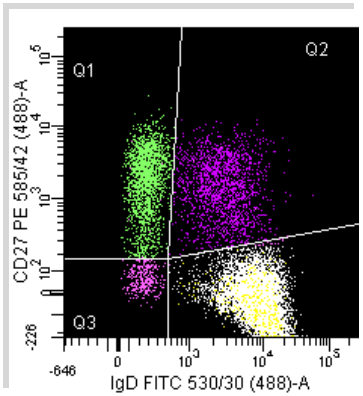
Validation Considerations Flow Cytometry

Validation Objective for Research-Use-Only (RUO), Lab Developed Test (LDT)

Establish method performance

- Specificity
- (Accuracy)
- Precision/Robustness
- Sensitivity/Limit of Detection
- Stability
- (Reference Intervals)

- Standard Calibrators
- Range of Quantification
- Stability
- Dilutional linearity
- Incurred Sample Reanalysis
- Interference (Matrix, Drug)
- Normal signal distribution
- Prozone effect



Flow Biomarker Assay

B cell Panel-4 Configuration

	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
	BV421	V500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
Gating Control	CD19	CD3/CD14/ CD56			CD20			CD45
EXP1	CD19	CD3/CD14/ CD56	IgD	CD27	CD20		CD69	CD45
EXP2	CD19	CD3/CD14/ CD56	CD138	CD24	CD20	CD38		CD45
EXP3	CD19	CD3/CD14/ CD56	IgD	CD27	CD20		CD10	CD45

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Flow Biomarker Assay

Reportable Results	
Population	**Phenotype**
CD19 B cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+
CD20 B cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD20+
Activated B cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+, CD20+, CD69+
Naïve B cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+, CD20+, CD27–
Memory B cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+, CD20+, CD27+
Short-lived plasma cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19dim, CD20-, CD27bright
Breg	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+, CD24bright, CD38bright
Plasma cells	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19dim, CD20-, CD138+
Plasmacytoid	CD45bright, SSClow, CD3-, CD14-, CD56-, CD20+, CD138+
Transitional	CD45bright, SSClow, CD3-, CD14-, CD56-, CD19+, IgD+, CD10+, CD27–

Specificity for Flow Biomarker Assays

Assay Design

- Fluorochrome usage vs. antigen expression
- mAb clone evaluation
- Reagent titration
- Matrix- cell lines, whole blood, PBMC
- Lysis, fixation, permeablization buffer selection
- Acquisition and analysis templates/gating strategy

Phenotype

- CD markers used to define the cellular population or antigens of interest must be justified or recent published data sought
- Commercial mAb specificity should be verified by the Leucocyte Differentiation Antigens Workshops or peer reviewed publication
- Novel/custom mAb specificity must be well documented internally

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Flow Biomarker Assay

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	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
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EXP3	CD19	CD3/CD14/ CD56	IgD	CD27	CD20		CD10	CD45

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Accuracy

Standard Definition

- closeness of the result compared to the true value of the analyte

GLP

- Determined by the mean bias determined in spiked recovery experiments

CAP/CLIA

- comparison to “gold standard” method
- measured concentrations in an official reference sample
- measuring a concentration in comparison to an official standard

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Accuracy for Flow Biomarker Assays

IVD

- CAP Proficiency Testing Surveys are available
- QC material with target values are available
- Inter-laboratory comparison

RUO/LDT

- Lack of proficiency testing programs
- Lack of cellular reference/QC material with target values for the populations of interest
- For novel or proprietary methods, sample exchange in not possible

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Precision for Flow Biomarker Assays

Precision

- Difficult to find samples with varying levels of each reportable result
- Weighted importance for biomarker data
  - Intended use of the data
  - Longitudinal, multicenter studies
  - Monitor responses due to treatment

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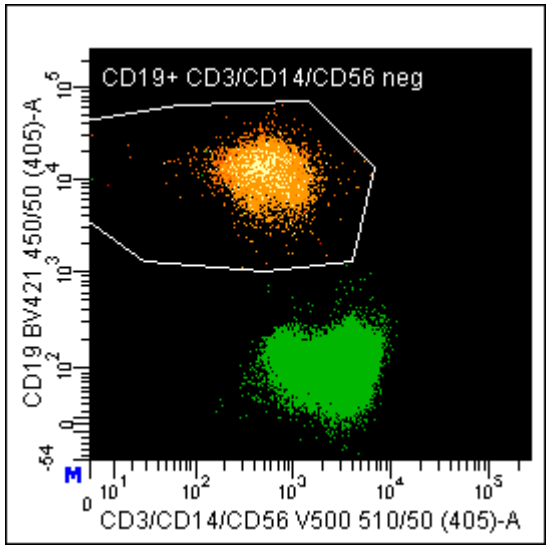
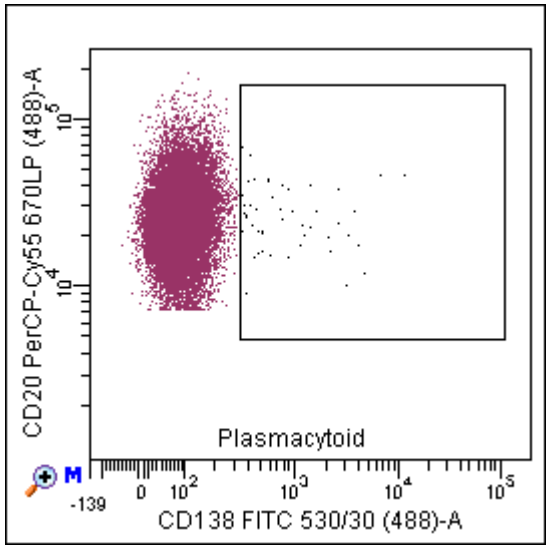
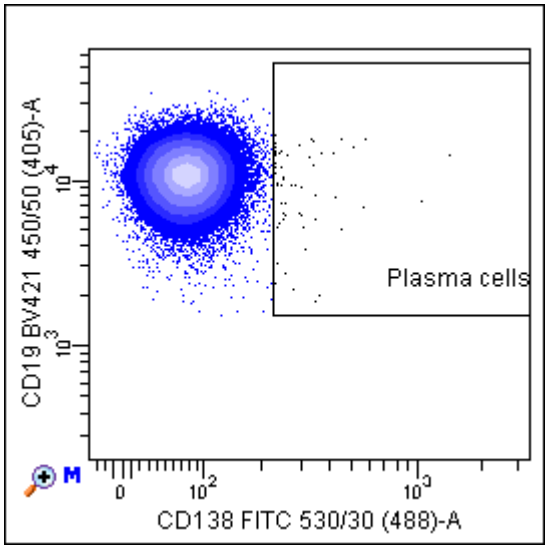
Precision for Flow Biomarker Assays

Acceptance Criterion

- <10 %CV desirable for all methods
- <20-25 %CV acceptable for immunoassays per Fit-for-Purpose paper
- <30 %CV may be acceptable for rare event detection use as exploratory biomarkers
  - With poor precision, more replicates and samples are required **(iterative approach!)**







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Sensitivity

Standard Definition

- the lowest reportable result

GLP

- lower limit of quantification (LLOQ) as the lowest concentration that can be measured with acceptable accuracy and precision (e.g., ± 20% CV)

CAP/CLIA

- response above the limit of detection (LOD)

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Sensitivity for Flow Biomarker Assays

Lower Limit of Detection (LOD)

- FMO controls

Gating Control	**CD19**	**CD3/CD14 /CD56**			**CD20**			**CD45**
EXP1	**CD19**	**CD3/CD14 /CD56**	**IgD**	**CD27**	**CD20**		**CD69**	**CD45**

Lower Limit of Quantitation (LLOQ)

- Difficult to find samples
- Mix stained and unstained samples
- Targeted cell depletion followed by re-spiking

Weighted importance for biomarker data

- Need to know at what point are the results are imprecise

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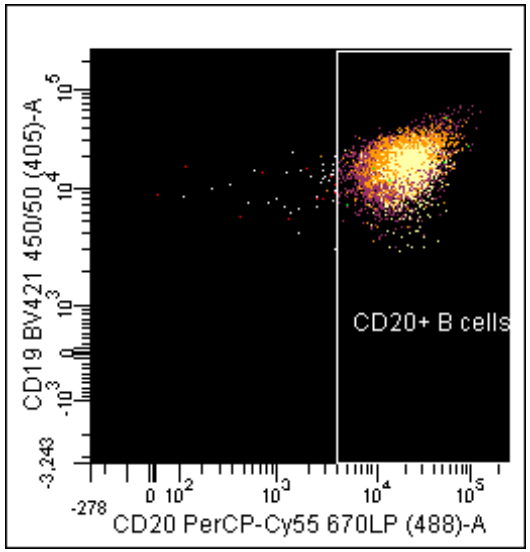
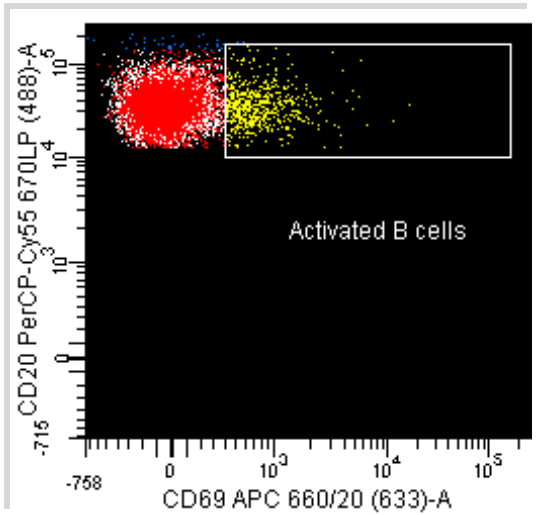
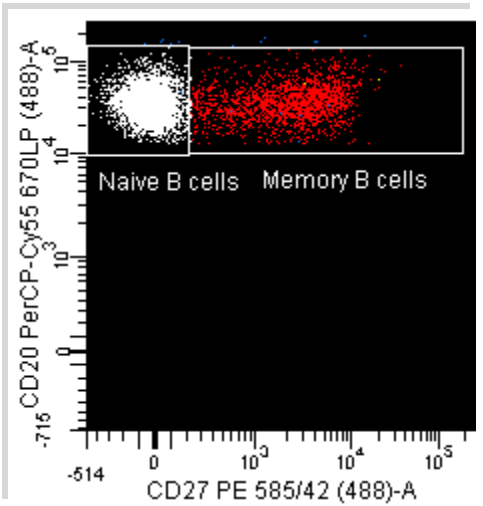
Reference Intervals for Flow Biomarker Assays

Reference Intervals

- Not required for first usage exploratory biomarkers, PD biomarkers
- Required for safety, diagnostic/disease biomarkers or companion diagnostics **(iterative approach!)**







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Challenges Related to Global Clinical Trials

Limited specimen stability

- Options are to use a central lab or multiple local labs
- Requires global standardization

Multicenter

- Data generated in multiple labs must be combinable for a final study data set
- Requires global standardization

Longitudinal Trials

- Greater emphasis on precision

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Flow cytometers and related software are used to support key decisions during the drug development process; validation of the system provides assurance that the data generated on these instruments is

reliable and precise

Overview of Presentation

- Key goals of system validation in drug development
- Unique challenges
- Approach to system validation in drug development
- Key phases of validation
  -Planning, Testing, Implementation
- Approach to installation, operation, performance qualification (IQ, OQ, PQ)
- Key concepts to managing life cycle of system after validation

Key Goals of System Validation

- Establish and maintain a controlled environment that can produce reliable data over a long period of time
- Ensure integrity and reconstruction of data
- Support lifecycle of the system by establishing procedures from installation to decommission

Unique Aspects of Flow Cytometry System Validation

- No accuracy standards
- Acquisition/analysis templates are customizable
- Final analysis may involve multiple software and LIMS
- Data consist of many outputs

  -Primary
  - Raw listmode files (.FCS)

  -Gated/Analyzed
  - Experimental data analysis templates (software specific)
  - Analyzed files using 3rd party software (.PDF, .PPT)
  - Statistics export (.XLS, .CSV, .TXT)
  - Post-acquisition calculations (compensation, calibration, counts)

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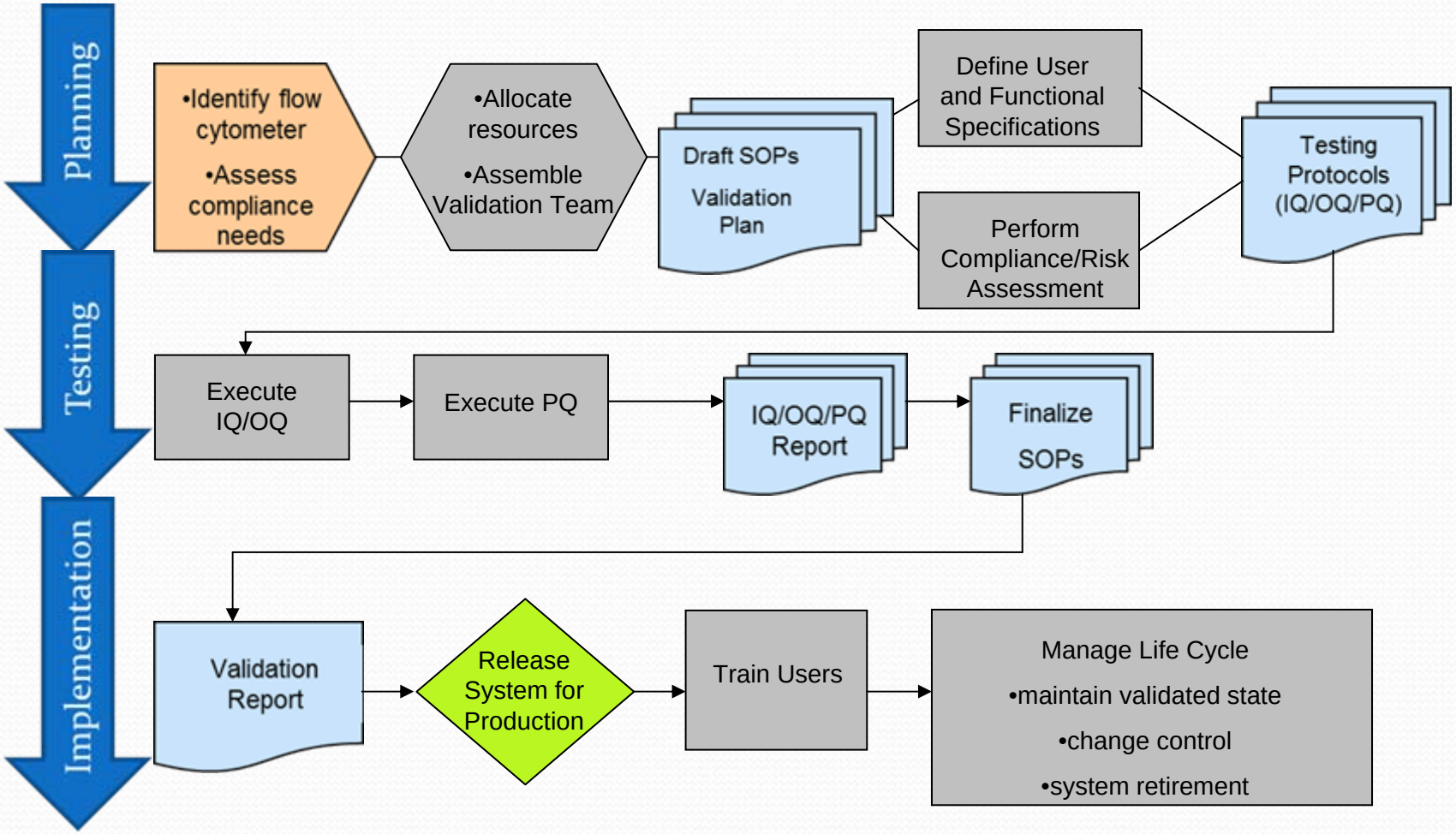
Approach to system validation in drug development

- Identify intended use and regulatory requirements of the system
  - Will the instrument be used to support discovery research, GLP pre-clinical studies, clinical studies, GMP lot release testing?
  - If system supports regulated testing, ideally system will be dedicated to this purpose.
  - Minimally, system should be properly installed with operational and performance characteristics verified with QC and change control system implemented
- Define scope- all elements of "the system" (instrument, software, data output)
- *Formal system validation, based on predicate rules (passing or failing as a measure of predefined criteria) can demonstrate the suitability of the instrument and ensure longitudinal integrity of the data*

Phases of Instrument Validation



Installation and Operational Qualification (IQ/OQ)

Purpose: Provide evidence that system is installed and functions per manufacturer's specifications AND user's requirements

Typical IQ parameters include:

- Environment (eg, space requirements)
- Utilities (temperature and electrical requirements, hardware and software)

Typical OQ parameters include:

- Software functionality
- Optical Precision
- Automated sample acquisition
- System alerts

Testing can be performed by vendor, qualified internal staff, or contracted external consultants

Performance Qualification

Purpose: Provide evidence that system performs for the intended purpose

- This can be the most time consuming part of validation
- Activities may overlap with assay validation
- Typical parameters include:
  - Instrument Performance
  - Inter-instrument comparison
  - Inter-laboratory comparison
  - Longitudinal performance

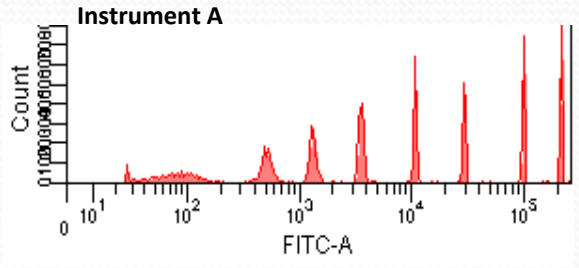
Testing is performed by qualified staff with system expertise

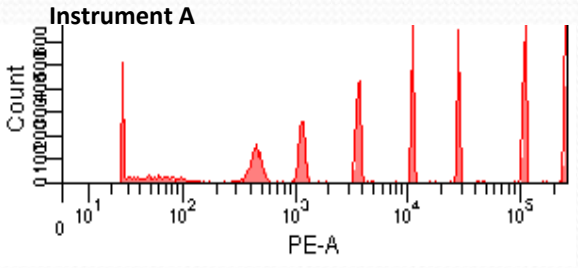
Instrument Performance

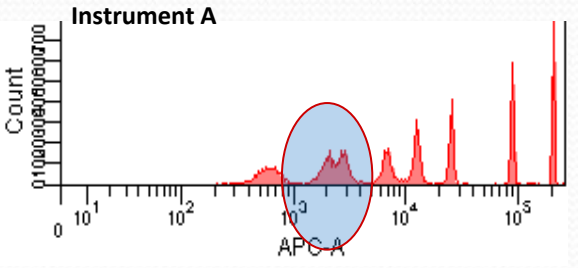
Stabilized biological material and calibration beads of different sizes and fluorescent properties may be used to assess:

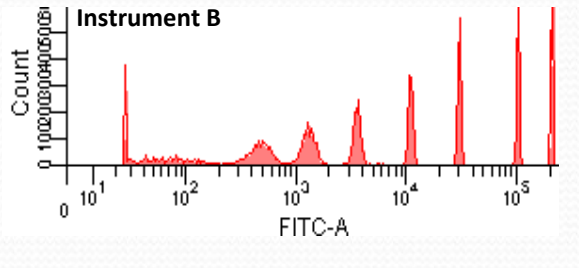
- Linearity
- Sensitivity
- Precision of acquisition
- Acquisition carry over
- Light scatter and fluorescence output

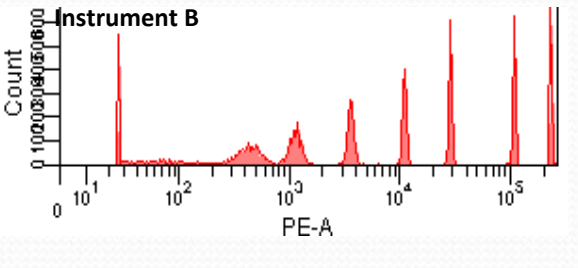
Multi-peak beads can assess sensitivity and linearity

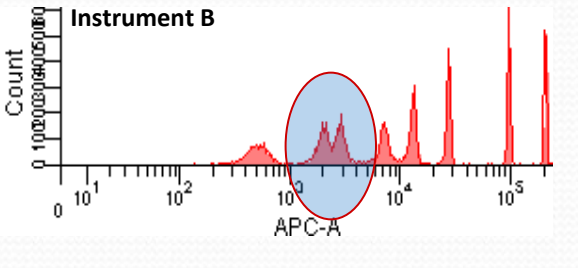


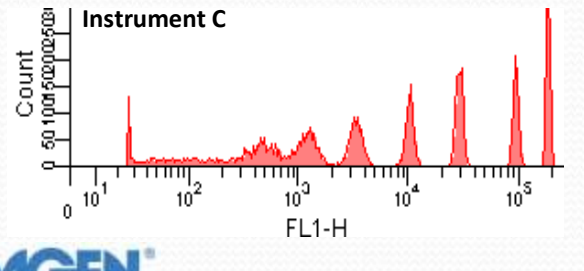


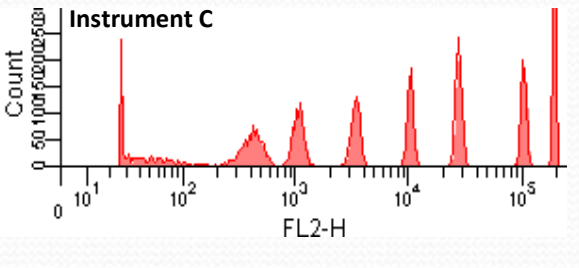


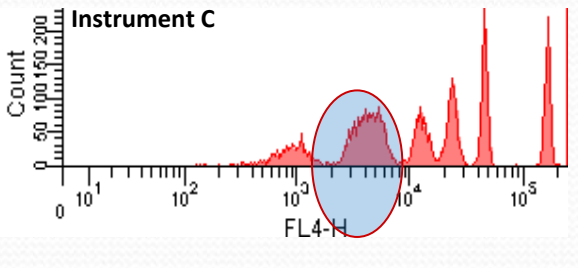












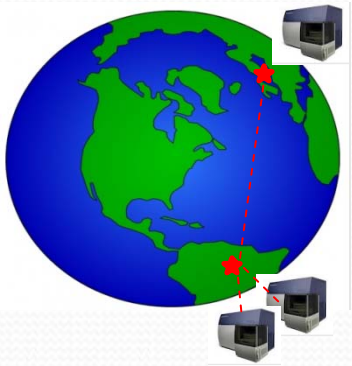
Inter-instrument Comparison

- Common and desired practice is to have backup instrument(s)
- Demonstrating comparability across instruments and apply same validation rigor
- Standardized procedures (SOPs) for instrument setup, calibration, and operation should be implemented to reduce variability
- Calibrated fluorescent beads can be used to establish target fluorescence output and standardize across platforms
- Establishing standardized, controlled acquisition and analysis templates can reduce variability
- Approaches to demonstrating comparability:
  - Parallel acquisition of samples

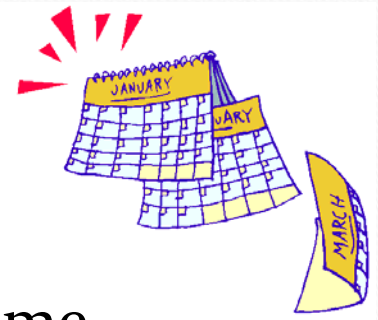
Inter-laboratory Comparison

Geographic requirements of clinical trials may require testing in many locations within a single trial

- Demonstrating comparability across instruments *and* sites is essential
  - Standardize fluorescent output
  - Cross-train analysts to harmonize practices
  - Perform site visits and/or web based training to ensure consistency in procedures
  - Standardize qualification of reagents at central location
- Approaches to demonstrating comparability:
  - Parallel processing and acquisition of samples
    - Sample stability may be problematic
    - Import/export issues
  - Control reagents [stabilized human blood or lyophilized cells]
    - May not work with custom assays



Longitudinal Performance



Quality performance must be maintained over time

- Especially true for long clinical trials which may take place over years
  - Example: measurements of protein levels or drug target occupancy will be compared back to pre-treatment values
- Fluorescence can be standardized by 2 approaches:
  - Generate a standard curve using particles of known fluorescence intensity [MESF; molecules of equivalent soluble fluorochrome]
  - Standardize fluorescence output by setting one [or more] bead peaks to a set channel number

Maintain Validated State Through Managing the System Lifecycle

- Continued review and update of SOPs
- Establish change control system
  - Identify change
  - Assign impact level
  - Determine need for change
- Re-validate, as needed
  - software and hardware upgrades
- System retirement

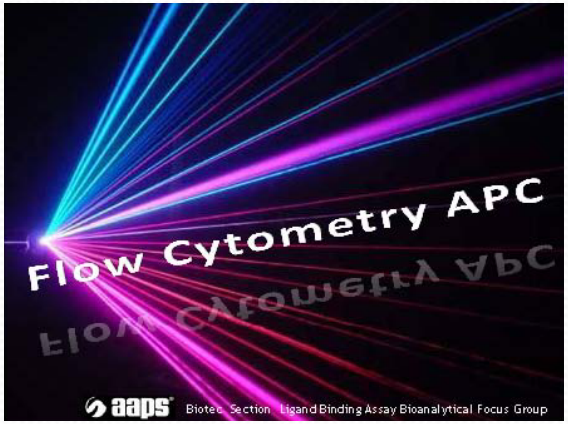
Final Thoughts:

System validation provides assurance that reliable data can be generated throughout the life cycle of the biomarker

- Assess and forecast the potential compliance needs of the biomarker
- Determine the depth of validation activities
- Implement appropriate procedures for verifying instrument and assay performance

Acknowledgements

AAPS, Biotec Section, Ligand Binding Assay Bioanalytical Focus Group ***Flow Cytometry Action Program Committee***



The group's mission is to promote discussion regarding the proper application of flow cytometry in drug development with an emphasis on establishing best practices regarding assay and instrument validation.

Steering Committee Past and Present

- **Virginia Litwin**
- **Cherie Green**
- John Ferbas
- Peter O'Brien
- Lynette Brown
- Sophie Corneau
- Kathy Howell
- Nicholas Jones
- Murli Krishna
- Zhyian (Eric) Lianz
- Thomas McCloskey
- Denise O'Hara
- Manjula Reddy
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- Christopher Wiwi
- Dianna Wu
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- Yuanxin Xu